#35

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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
HELDIN et al.
Serial No.: 08/453,350
Filing Date: May 30, 1995
Title: RECOMBINANT PDGF A-CHAIN HOMODIMERS AND METHODS OF USE

Group Art Unit: 1812
Examiner: K. Brown

Declaration of Christer Betsholtz, Ph.D.

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Christer Betsholtz, declare as follows:

1. I am a coinventor of the above-identified application. I hold a Ph.D. in Experimental Pathology from the University of Uppsala, Sweden. I am employed by the Department of Medical Biochemistry at the University of Gothenburg, Gothenburg Sweden, as a Professor of Medical Biochemistry and have held this position since 1993. Prior to that time, I was a Senior Research Fellow at the Cancer Research Fund, Sweden and held Post doctoral positions at the Cancer Research Fund and the Medical Research Council, Sweden. A copy of my *curriculum vitae* is attached hereto as Exhibit A.

2. I am extremely familiar with PDGF and have actively been studying this molecule, including the molecular characterization and mechanisms of action thereof, for over 15

years. I have coathored numerous publications regarding PDGF. A true and correct copy of my *curriculum vitae* is attached hereto as Exhibit A.

3. I have reviewed the Office Action dated July 8, 1997 ("the Action"), as well as Heldin et al., *Nature* (1986) 319:511-514 ("Heldin"), cited in the Action, on which I am a coauthor. In the Heldin paper, we describe the isolation of what was termed therein osteosarcoma-derived growth factor ("ODGF"). We now know this molecule to be the same as a naturally occurring PDGF A-chain homodimer. However, at the time of the publication, we did not realize that this molecule was the same as a naturally occurring A-chain homodimeric form of PDGF because we did not know that such a form of naturally occurring PDGF existed (see, page 513, second column, of Heldin).

4. The ODGF described in Heldin was isolated directly from a human osteosarcoma cell line using sequential chromatography of conditioned medium from U-2 osteosarcoma cells. This cell line was established from a human patient suffering from cancer. Although this cell line was propagated in culture, the cell line may well have contained pathogenic viruses from the patient. Moreover, since the cell line was of human origin, it could easily become infected with human pathogenic viruses during propagation. Therefore, I believe the Action's statement on page 4 that the ODGF "would be highly unlikely to be contaminated with virus" to be in error. The methods used to purify ODGF described in Heldin would not guarantee the elimination of human viruses. This method would not be appropriate for producing an ODGF compound to be used in pharmaceutical compositions since viral contaminants may be present. Accordingly, there would be a high

risk associated with using ODGF purified as described in Heldin, in compositions for treating patients.

5. Additionally, the methods described in Heldin would not produce ODGF preparations completely free of other human proteins. In particular, ODGF was isolated using a Sephacryl S-200 column, followed by a BioGel P-150 column, and then an HPLC RP8 column. The product of each of these methods would inherently include at least small amounts of human proteins other than human ODGF since the ODGF was isolated from human osteosarcoma cells. The methods for purifying proteins from human sources described above, cannot result in a protein product free of contaminating human proteins. The Action notes that amino acid sequence analysis and silver staining are highly sensitive methods for determining whether a protein sample is homogeneous. Despite these observations, it is a virtual certainty that trace amounts of human proteins were present in the ODGF preparations that were not detected by these methods.

6. Recombinant methods of producing human PDGF A-chain, such as those described in the subject application, on the other hand, result in a preparation free of other human proteins and devoid of contaminating human viruses. This is because the only human structural gene present in the recombinant plasmids is the gene encoding human PDGF. It would not be possible to produce preparations having such purity without the gene encoding PDGF. Heldin does not describe the gene or recombinant methods for producing PDGF A-chain.

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7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

98-02-11
Date

Christer Betsholtz, Ph.D.

EXHIBIT A

CURRICULUM VITAE

CHRISTER BETSHOLTZ

Born: July 11, 1959 in Stockholm, Sweden.

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Citizenship: Swedish

Family Married to Ingrid Holmberg-Betsholtz, two children born 1988 and 1990

Degrees and appointments (in chronological order):

1980	Bachelor of Medicine, University of Uppsala
1986	PhD
1986-88	Post doctoral position (TheCancer Research Fund, Sweden)
1989-90	Post doctoral position (The Medical Research Council, Sweden)
1991-93	Senior Research Fellow (The Cancer Research Fund, Sweden)
1993-	Professor of Medical Biochemistry, Gothenburg University, Sweden

Scientific awards

1989: The Oscar Prize, Uppsala University
1995: Fernström's Prize for young investigators, Gothenburg University
1997: Göran Gustavsson's Award in Molecular Biology

Scientific projects

Genetic analysis of platelet-derived growth factor functions.
Islet amyloid polypeptide (amylin) molecular genetics.
Glial fibrillary acidic protein, intermediate filaments and astrocyte functions.

Invited lectures at international sympodsia (from 1994-1997, selected):

Keystone symposium "Biology of the vascular wall/endothelial cells" Keystone, CO 1994
Second Nordic workshop on transgenic mice, Turku, Finland 1994
Reaction to injury revisited, Univ of Washington Symposium, Seattle, WA 1994
Tsumagoi Conference on cardiovascular biology, Kyoto, Japan, 1995
International Vascular Biology Meeting, Seattle, WA 1996
ISN Forefronts in Nephrology, Snowbird, Utah, 1996
Keystone symposium "Inflammation, Growth regulatory molecules and Atherosclerosis", Keystone CO 1997
Cold Spring Harbor symposium on Signal transduction in endothelial cells 1997

1998: Gordon Conference on vascular biology, July 1998
Juselius Symposium on angiogenesis, Helsinki June 1998
FEBS Silver Jubilee meeting, Copenhagen July 1998

Morphogenesis of the endothelium, Schloss Ringberg Germany, September 1998
7th International workshop Developmental Biology, Stockholm September 1998
5th Franz-Volhard Symposium, Berlin Germany, September 1998

Ad-hoc reviewer for:

Atherosclerosis
Cancer Research
Development
Diabetologia
European Journal of Biochemistry
Experimental Cell Research.
FEBS Letters
Growth Factors
International Journal of Cancer
Journal of Biological Chemistry
Journal of Cell Biology
Nature
Nucleic Acids Research
The Cancer Journal

Scientidic publications (selected)

Betsholtz, C., Heldin, C.-H., Nistér, M., Ek, B., Wasteson, Å. and Westermark, B. Synthesis of a PDGF-like growth factor in human glioma and sarcoma cells suggests the expression of the cellular homologue to the transforming protein of simian sarcoma virus. ***Biochem Biophys Res Commun*** 117,176-182 (1983)

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localisation of human platelet-derived growth factor A-chain and its expression in tumour cell lines. *Nature* 320,695-699 (1986)

Johnsson, A., Betsholtz, C., Heldin, C.-H. and Westermark, B. The phenotypic characteristics of simian sarcoma virus-transformed human fibroblasts suggest that the v-*sis* gene product acts solely as a PDGF receptor agonist in cell transformation. *EMBO J* 5,1535-1541 (1986)

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